Concept Review:

Analytical Chemistry for the Environmental Toxicology Program

NIEHS
The National Institute of Environmental Health Sciences
The National Institutes of Health
The Department of Health and Human Services

NTP
The National Toxicology Program
The Department of Health and Human Services

Why is Analytical Chemistry Important in Bioassays?

Bioassay Paradigm

Exposure	Internal Dose	Response
Test article and formulation analysis	Blood/Tissue concentration and/or biomarkers	Toxic Effects

Response must be supported with good analytical chemistry data for exposure and internal dose.

Classes of Test Articles Studied by the NTP

Drinking Water Contaminants - BDCM, DCA, Bromopicrin

Food Additives and contaminants – Methyleugenol, hydroxymethylfurfural, 2- and 4-Methylimidazole

Materials used in making plastics - bis(2-Chloroethoxymethane), Dimethyl-p-toluidine, Formamide

Flame Retardants – TBBPA, PBDEs

Consumer Product Ingredients – Dibromodicyanobutane, Chitosan

Metals - Hexavalent chromium, Chromium picolinate

Pharmaceuticals - AIDS Combination Therapies, Elmiron

Solvents - MIBK, Tetralin, Stoddard Solvent

Botanical Products - Ginkgo biloba extract, Goldenseal root

Blasting Agents - Blasting sand, Garnet, Crushed glass, Coal slag

Overview of the Chemistry Role

Types of studies supported:

- Carcinogenicity
- General Toxicology
- Reproductive Toxicology
- Immunotoxicology
- Genetic Toxicology
- Differential Gene Expression
- DIR in-house research upon request

Overview of Chemistry Role (cont'd)

Types of tasks performed:

- Chemical Procurement
- Chemical Characterization
- Dose Formulation Development
- Biological Sample Analysis
- Toxicokinetics Studies with Unlabeled Compounds

Capabilities

Chemical Characterization:

- Physical Constants Determination
- IR, NMR, MS, MS/MS
- Elemental Analysis
- Water Determination
- Chromatographic Analyses - HPLC, GC, IC,TLC – all detectors
- Functional Group Titration
- Storage Stability Evaluation

Chemical Characterization for Bioassays

Unequivocal Identity

- IR, NMR, MS
- Physical Constants (Chronic only)

Purity Determination

- Water Determination
- Elemental Analysis (Chronic only)
- 2 Orthogonal Chromatographic Analyses (Organics, ICP/AES or ICP/MS for Inorganics)
- Impurity identifications at ≥ 1 %
- Impurities reported at ≥ 0.1 %

Preliminary Chemistry Studies

- Solubility
- Suspendability
- Palatability
- Gavageability
- Inhalation Feasibility

Dose Formulation

- Vehicle Characterization
- Dose Analysis Method Development and Validation
- Homogeneity Evaluation
- Stability Studies

Toxicokinetics Studies

- Unlabeled test article
- Pilot study – feasibility, when needed
- Preliminary study - IV and bioassay route
- Initial doses based on literature values of LD_{50}
- Tissues used to develop bioanalytical method
- Definitive study - GLP with multiple samples/time point
- Single animal data QC'd spreadsheets => modelers for PBPK
- Results => parameter values from non-compartmental model

Typical Non-compartmental Parameters

Values are reported for typical parameters including:

- AUC
- AUMC
- Cl
- F
- MRT
- t
- $\bar{V}$
- k

New to this SOW

Current impurities paradigm = Identify >1 %; report >0.1 %

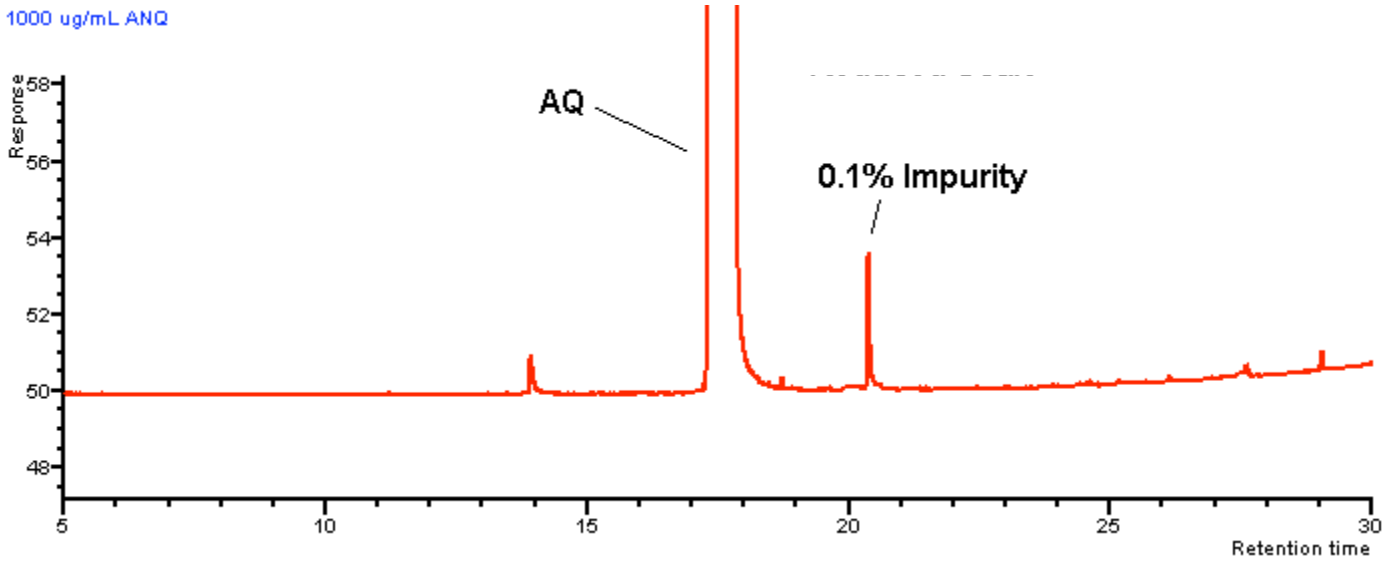
Some studies require more:

New Assignment – Low Level Impurity Determination (LLID)

- Offline from routine characterization
- Iterative plan with interim data submitted
- High priority

Example: Anthraquinone

Anthraquinone Purity Analysis by GC/FID

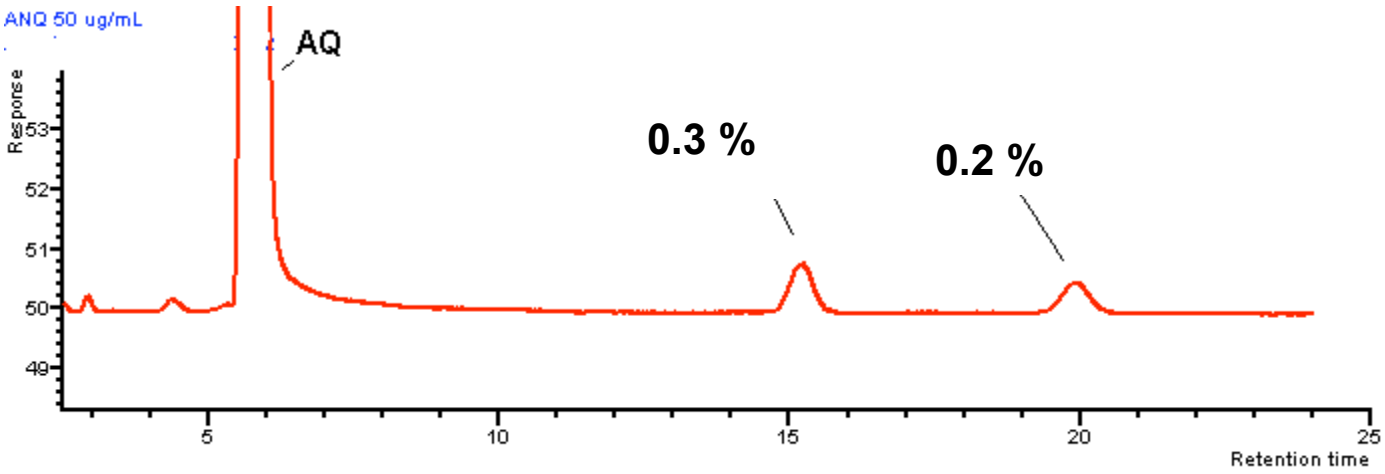


Initial analysis by GC/FID - 0.1 % impurity

Others - smaller and therefore not reported

Overall purity = 99.9 %

Anthraquinone Purity Analysis by HPLC/UV



Initial analysis by HPLC/UV – reported impurities were 0.2 and 0.3 % of total peak area

Others - 0.01 % and 0.03 % not reported

Overall purity = 99.5 %

Impurity Questions

In response to concerns of stakeholders:

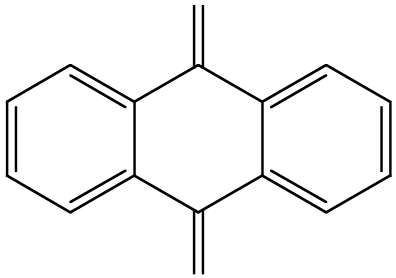
1) Impurities identified

2) Rationale - difference between GC purity and HPLC purity

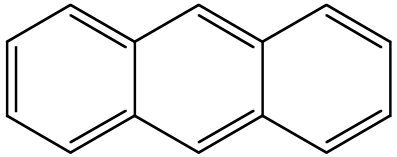
3) Impurities quantitated

Impurity Questions 1) and 2)

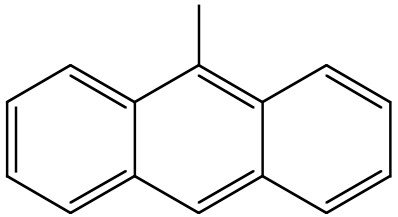
1) HPLC/MS showed the presence of 5 compounds:



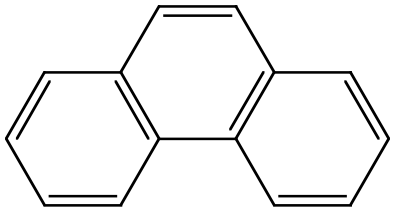
Anthraquinone



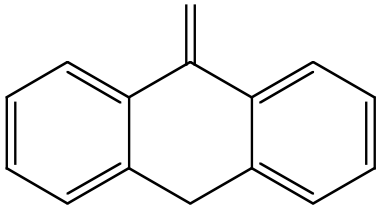
Anthracene



9-Nitroanthracene



Phenanthrene

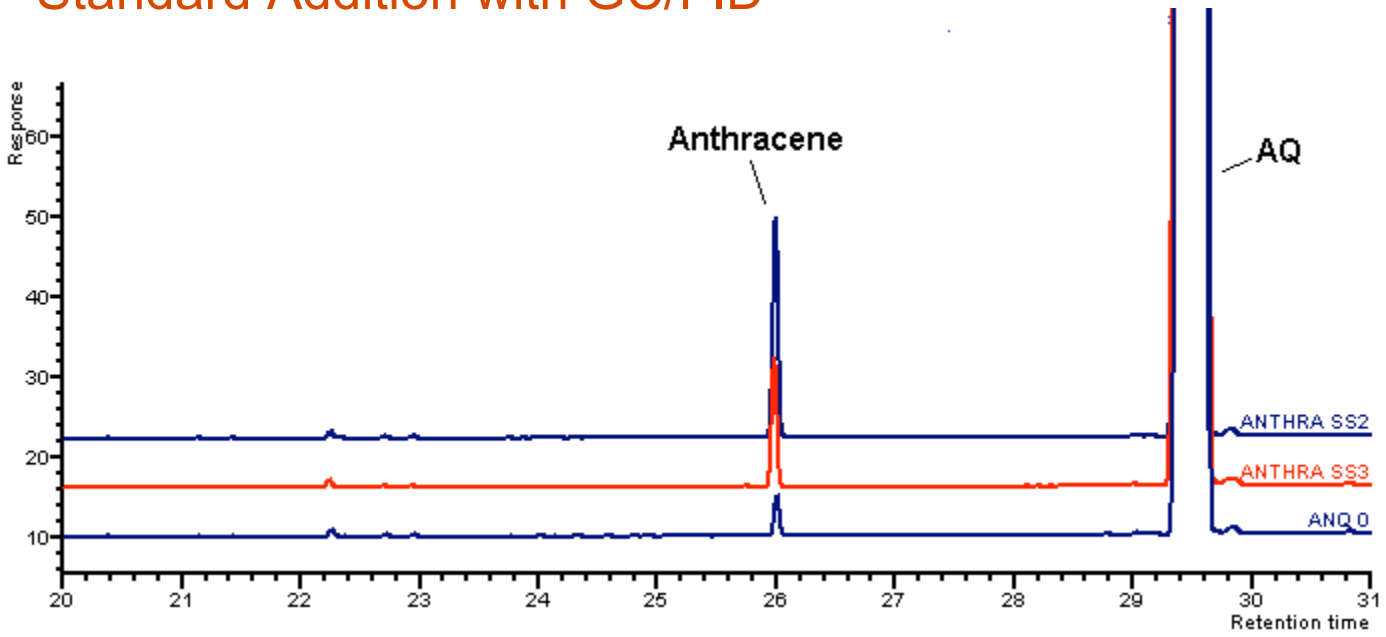


Anthrone

2) Ultraviolet absorbance roughly doubles with each conjugated double bond

Impurity Question 3)

Anthracene Impurity Quantitation by
Standard Addition with GC/FID

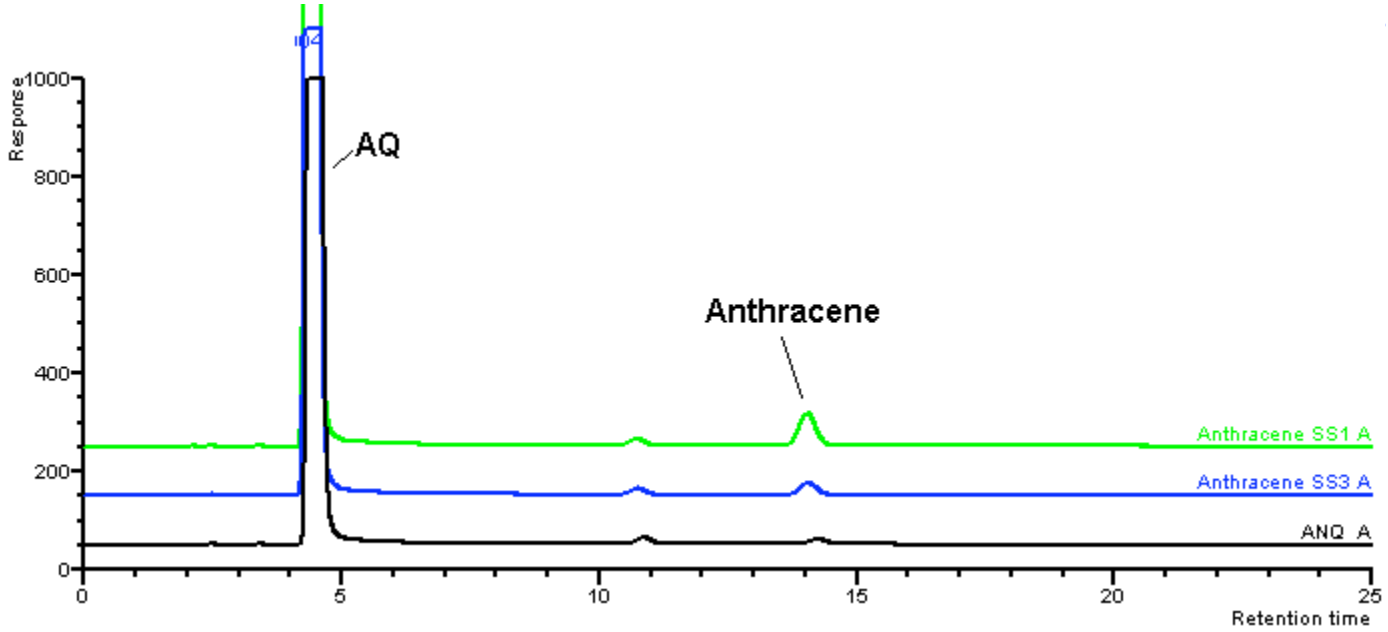


Anthracene = 0.05 %
9-Nitroanthracene = 0.1 %
Phenanthrene, Anthrone = < 0.002 %

Overall purity = 99.85 %

Impurity Question 3) cont'd

Quantitation of Anthracene Impurity with HPLC/UV
By Standard Addition



Anthracene = 0.06 %
9-Nitroanthracene = 0.11 %
Phenanthrene, Anthrone = < 0.001%

Overall purity = 99.83 %

New to this SOW

New assignment – Chemical Identity and Purity Screen (CIPS)

Acceptable techniques will be:

- Automated
- Easily interpreted
- Cost effective

Chief techniques will be NMR (organics) and ICP/AES (inorganics). Fall-backs will be Flow-injection or Direct Probe MS and HPTLC

New to this SOW

New Assignment – Biochemical Measurement (BCM)

Intended as an interim measure.

Assignments will be:

- documented in the literature
- well accepted in the research community
- require no method development
- biochemical measurements

Examples – chemical biomarker measurements, enzyme assays, protein binding assays

Expectations for Procurement

- 3 awards projected
- 800 assignments/year/award
- Full range of capabilities/award

Cost Containment

1. Use test articles/methods in multiple programs wherever possible

2. Use high throughput analyses for test articles intended for high thoughput studies

3. Place emphasis on reporting interim data to direct work in a facile timeframe